PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/007529	08.07.2004	08.07.2003

International Patent Classification (IPC) or both national classification and IPC
C12N5/06, C12N15/79, C12N15/82, C12Q1/68

Applicant
AXIOGENESIS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/007529

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 44 (for I.A.)

because:

☒ the said international application, or the said claims Nos. 44 (for I.A.) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 4, 8, 9, 11, 12, 15, 18, 19, 21, 23-31, 37-44, 48, 52 and 53
	No:	Claims	1, 2, 5-7, 10, 13, 14, 16, 17, 20, 22, 32-36, 45-47, 49-51 and 54
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-54
Industrial applicability (IA)	Yes:	Claims	1-43, 45-54
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 37, 38, and 43-47 lack clarity (Article 6 PCT) because the products to which the claims relate are not defined by characteristic features but by a method which should lead to their identification leaving therefore the reader in doubt as regards the intended scope of protection. Moreover the description is silent as to a potential common or essential characteristic feature for the claimed product. The lack of clarity is such that the claims could not be searched over their entire scopes and the search and therefore the opinion as regards novelty, inventive step and industrial applicability (Article 33 PCT) for the subject-matters of claims 45 and 46 have been limited to the compounds and pharmaceutical compositions clearly defined in the description as promoting or modulating cell growth and/or differentiation, i.e. retinoic acid and pharmaceutical compositions comprising the same (see the examples). Moreover claims 37 and 38 do not provide any steps for the manufacture of the drug the claims relate to leading therefore to a lack of clarity (Article 6 PCT).

Since the features following the expressions "for example", "like" in claim 42 are considered as non limiting features for the subject-matter of said claim (cf. PCT International Search and Preliminary Examination Guidelines 5.40), claims 42 relates to a kit which is only defined by a result to be achieved "useful for conducting the method of claims 1 to 12 or 23 to 41". Therefore claim 42 lacks clarity (Article 6 PCT) to such an extend, that a meaningful search for claim 42 on its entire scope could not be performed.

Moreover the expression "standard compounds" is not clear (Article 6 PCT) for the following reasons: i) this expression does not have any commonly well accepted definition and ii) the description is also silent as regards its definition.
Therefore the search and consequently the establishment of a preliminary opinion as regards novelty, inventive step and industrial applicability (Article 33 PCT) for the subject-matter of claim 42 has been limited to kits containing a reporter gene construct of claim 13, or a cell of claim 14 and the standard compounds specifically cited in claim 42 after the term "like" - i.e. culture media, selection agents, detection agents for the reporter molecule and control samples.

Claim 44 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iii) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of said claim (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents D are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: US 2003/008836 A1 (Goldspink Geoffrey) 9 January 2003

D2: Müller M. *et al.* - "Selection of ventricular-like cardiomyocytes from ES cells *in vitro*" - 2000 - *Faseb J.*, **14**: 2540-2548

D3: Wobus A. M. *et al.* - "Retinoic acid induces expression of the ventricular 2.1 kb myosin-light-chain2 promoter during *in-vitro* cardiogenesis of embryonic stem cells" - 1995 - *Circulation*, **92**(8): I-114

D4: Wobus A. M. *et al.* - "Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes" - 1997 - *J. Mol. Cell. Cardiol.*, **29**: 1525-1539

D5: Klug M. G. *et al.* - "Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts" - 1996 - *J. Clin. Invest.*, **98**: 216-224

D6: Bronstein I. *et al.* - "Chemiluminescent reporter gene assays: sensitive detection of the GUS and SEAP gene products" - 1994 - *Biotechniques*, **17**: 174-177

D7: US 2002/042096 A1 (Rosen Craig A. *et al.*) 11 April 2002

2. Novelty

2.1 The present application does not meet the requirements of Article 33(1) PCT, because the subject-matters of claims 1, 2, 5-7, 10, 13, 14, 16, 17, 20, 22, 32-36, 45-47, 49 -51

and 54 are not new in the sense of Article 33(1) and (2) PCT.

2.1.1 Document **D1** discloses a method allowing the follow-up of cell differentiation wherein undifferentiated myoblast are transfected with vectors driving the expression of human alpha-gal which expression is under the control of Rabbit β-cardiac myosin heavy chain (MHC) (cf. **D1** [0053] and table 1). The expression of the target gene alpha-gal which is secreted in the culture medium can be measured at various time points during the differentiation process in order to monitor said differentiation of myoblast into mature myotubes (cf. **D1** [0053] - [0057]).

Hence document **D1** anticipates the method of claims 1, 2, 5-7, 10, 32-36, the reporter gene construct of claim 13, the cell of claim 14, the tissue and organ of claims 16 or 17, the composition of claim 20, and the vector of claims 49 and 51. Thus claims 1, 2, 5-7, 10, 13, 14, 16, 17, 20, 32-36 and 49 do not meet the requirements of Article 33(2) PCT.

2.1.2 Documents **D2**, **D3** and **D4** disclose vectors comprising the promoter region of the ventricular-specific myosin light chain-2v linked to a heterologous DNA sequence encoding the enhanced green fluorescent protein (cf. **D2** abstract, p. 2541 right-hand column 1st §, p. 2542 right-hand column last § - p. 2543 left-hand column 1st §; fig. 1 and 2; **D3** abstract and **D4** p. 1527 left-hand column 2nd §).
Also Document **D5** describes a vector comprising the promoter region of the α-cardiac myosin heavy chain (α-MHC) linked to the gene encoding aminoglycoside transferase (Neo^r) (cf. **D5** p. 216 right-hand column last § - p. 271 left-hand column 1st §).
Therefore documents **D2**, **D3**, **D4** and **D5** anticipate the subject-matter of claims 49-51 and 54 which, thus, do not meet the requirements of Article 33(2) PCT.

2.1.3 A microscope stands as an aparatus suitable for analyzing the array of claim 21 and therefore anticipates the novelty of present claim 22 (Article 33(2) PCT).

2.1.4 Retinoic acid is a well known inducer of cell differentiation (cf. **D4** p. 1527 paragraph bridging left to right-hand columns - p. 1529 left-hand column 1st §, and Fig. 1) which can be identified by the claimed methods, therefore, compositions comprising retinoic acid and the use of said compositions anticipate the novelty of claims 45-47 (Article 33(2) PCT).

2.2 No document at hand discloses:

- the additional features brought forward in the method of claim 1 by dependent claims 3, 4, 8, 9, 11, 12;
- the cell aggregate of claim 15;
- the implant of claim 18;
- the non-human animal of claim 19;
- the array of independent claim 21;
- the method of claims 23-31 and 37-41;
- the kit of independent claim 42;
- the method of independent claims 43 and 44;
- the use of claim 48;
- the vector of claims 52 and 53.

The subject-matters of claims 3, 4, 8, 9, 11, 12, 15, 18, 19, 21, 23-31, 37-44, 48, 52 and 53 are therefore novel, said claims thus meet the requirements of Article 33(2) PCT.

3. Inventive step

3.1 The subject-matters of claims 3, 4, 8, 9, 11, 12, 15, 18, 19, 21, 23-31, 37-44, 48, 52 and 53 are not considered to meet the requirements of Article 33(3) PCT because of lack of inventive step, the reasons being as follows:

3.1.1 The type of cells used in the method of claim 1 is considered to be an obvious option the skilled person will select without exercise of inventive skills, and the

formation of aggregates or embryoid bodies which only depends on the cell type chosen is thus not considered inventive either (relevant for claims 3 and 4; 11, 12 and 15);

3.1.2 Document **D4** is considered to be the closest relevant prior art for claim 9 and discloses a method for the monitoring of cell differentiation. Said method includes the following steps (cf. **D4** p. 1527 paragraph bridging left to right-hand columns - p. 1529 left-hand column 1st §, and Fig. 1):

- culturing embryonic stem (ES) cells capable of differentiating and forming embryoid bodies, which contain at least one recombinant nucleic acid molecule comprising a reporter gene encoding a specific and detectable product upon cell differentiation (i.e. lacZ gene under the control of MLC-2v promoter region), under conditions allowing differentiation of the cells (i.e. retinoic acid); and
- determining the amount or activity of the reporter gene product.

The method of claim 9 differs from the method of document **D4** by the fact that the reporter gene encodes a product that is secreted.

In view of **D4**, the problem to be solved by the method of claim 9 can be considered as the provision of an improved method for the detection and quantification of cells undergoing differentiation.

The solution to said problem, as contained in claim 9 is the use of a different reporter gene which product is released in the culture medium such as secreted alkaline phosphatase (SEAP) or alpha-amylase as mentioned in claim 9. Because document **D6** disloses all the advantages of using a SEAP as a reporter gene: i) the cell population remains intact since the detection is performed on the culture supernatant; ii) the detection of SEAP is quantitative and iii) highly sensitive especially when detected with a chemiluminescent assay (cf. **D6** p. 172 right-hand column last § - p. 173 left-hand column 1st §, p. 174 paragraphe linking the left-and the right hand columns, Fig. 2 , results and discussion), the skilled person wishing

to solve the aforementioned problem would turn to document **D6**.

Moreover, document **D7** further supports the use of SEAP reporter system and describes a reporter assay wherein the reporter gene (secreted alkaline phosphatase) is put under the control of different promoters and enhancers, the choice of said promoter / enhancer depending on the event to be monitored (cell activation, proliferation and differentiation)(cf. **D7** examples 32-36).

The examining division considers that the skilled person having knowledge of **D4** and **D6** would have solved said technical problem without exercise of an inventive skill. Thus claim 9 does not fulfill the requirements of Article 33(3) PCT.

3.1.3 the promoters or enhancers proposed in dependent claim 8 are well known in the art and the choice of a specific promoter or enhancer is a common design procedure for the person skilled in the art of vector for reporter assays (relevant for claim 8);

3.1.4 No inventive skills are required to transfert the already known organ or tissue of claim 17 or 16 respectively (see point 2.1 above) into another animal, for said organ or tissue to become a transplant or implant, therefore the subject-matters of claim 18 and 19 do not rely on inventive skills;

3.1.5 Multiple well cell-culture plates are well known in the art and would be an obvious option for culturing the cells, cell aggregates or tissues of the present invention (relevant for claim 21);

3.1.6 Document **D4** is also considered to be the closest relevant prior art for claim 23 and discloses a method for the detection or the characterisation of a modulator of cell differentiation. Said method includes the following steps (cf. **D4** p. 1527 paragraph bridging left to right-hand columns - p. 1529 left-hand column 1st §, and Fig. 1):

- contacting a test sample comprising a cell capable of differentiating, which

contains at least one recombinant nucleic acid molecule comprising a reporter gene encoding a specific and detectable product upon cell differentiation (i.e. lacZ gene under the control of MLC-2v promoter region) with a test substance (i.e. various form of retinoic acid); and

- determining the effect of the test substance on the amount of the reporter gene product or activity compared to a control sample.

The method of claim 23 differs from the method of document **D4** by the fact that the reporter gene encodes a product that is secreted.

By the same reasoning as regards the subject-matter of claim 9 (cf. point 3.1.2 above), in view of **D4** and **D6,** the method of independent claim 23 is not considered inventive and claim 23 does not fulfill the requirements of Article 33(3) PCT;

3.1.7 In the art of highthroughput or hit hunting, the use of single or plural, known or unknown, compounds as the test substance is well known and similarly their use in the hereinabove method (cf. point 3.1.2 and 3.1.6) is considered to be an obvious options for the skilled person (relevant for dependent claims 24-30); the performance of such assay in highthroughput format is a well known "must be" in the art of hit hunting, which therefore does not require inventive skills (relevant for claim 31) and the use of compounds derivatives presenting altered physical properties as well as compounds in pharmaceutical carrier is also an obvious option the person skilled in hit hunting would select without exercise of inventive skills (relevant for claims 40 and 41);

3.1.8 The methods of independent claims 37-39 are equivalent to the method of independent claim 23, with however the addition in the wording of said claim of a result to be achieved by said method. Since, said result to be achieved is solely related to the "test substance" used in the said method, it does not imply an intrisic, particular technical feature of the method which is therefore identical as that of claim 23. Therefore for identical reasons as those exposed as regards the lack of

inventive step in the subject-matter of claim 23 (cf. point 3.1.6 above), the subject-matters of claims 37-39 lack also inventive step contrary to the requirements of Article 33(3) PCT.

3.1.9 In the light of the objections put forward above, for the person skilled in the art, grouping the materials used for a series of known or obvious experiments in the form of a kit is not inventive. Thus, claim 42 does not meet the requirements of Article 33(3) PCT.

3.1.10 Document D1 is considered to represent the closest prior-art for the method of independent claims 43 and 44. The method of claims 43 and 44 differ from the method of document D1 by the steps delineated in subparagraph (a) and (b) in the claim. However, said steps (a) and (b) represent comon steps undertaken by skilled person or even companies in order to develop drug or target discovery business. Therefore the said steps cannot be taken into consideration when assessing the inventivity of the methods, and claims 43 and 44 are considered not inventive (Article 33(3) PCT) for identical reasons as those exposed to demonstrate the lack of inventive step of the method of claim 23 (see point 3.1.6 above);

3.1.11 The use of reporter gene or cells, cell aggregates or tissue containing reporter genes in drug discovery or pharmacokinetic is well known in the art of new drug development. Since the reporter gene or the cells, cell aggregates or tissue to which the use of claim 48 refers to are not considered novel or inventive, an inventive step for the use of claim 48 cannot be acknowledged either (Article 33(3) PCT);

3.1.12 In view of our previous objections as regards lack of inventive step for the use of secreted protein as reporter labels (see points 3.1.2 and 3.1.6), it appears that the vectors of claims 52 and 53 do not solve any particular technical problem or lead to any supprising or unforseeable technical effect. Therefore an inventive step for the subject-matters of the said claims cannot be acknowledged (Article 33(3) PCT.

4. Clarity

4.1 Independent claims 13-21, 42, 45, 46 are unclear (Article 6 PCT) because the products to which said claims relate to are defined by reference to method claims and not by characteristic features of the products themselves, therefore leaving the reader in doubt as to the real scope of the claims (see also PCT International Search and Examination Guidelines 5.31-5.33).

4.2 The number of independent claims is too high (23 independent claims) wherein independent claims 1, 23, 37-39, 43 and 44 relate to highly similar methods leading therefore to a lack of clarity of the entire set of claims (Article 6 PCT; see also PCT International Search and Examination Guidelines 5.42).

4.3 Claims 37-39 are unclear (Article 6 PCT) because they define independent methods which however are identical to the method of independent claim 23 (identical steps) and only differ by respective results to be achived which in turn, depend completely on the properties of the compound which is to be identified by the said method (see also PCT International search and Examination Guidelines 5.35).

4.4 The features following the expression "for example" in claim 42 has no limiting effect on the scope of said claim (see also PCT International Search and Examination Guidelines 5.40) and claim 42 therefore does not contain any features characterizing the kit its relates to. Claim 42 therefore lacks clarity (Article 84 EPC) and its examination as to novelty and inventive step (cf. above) has been performed insofar as if the expression "for example" was deleted from the claim.

4.5 Claim 43 lacks clarity (Article 6 PCT) due to the fact that its preamble relates to method of conducting business whereas the steps of said methods are not of business related type.

5. Industrial applicability (Article 33(4) PCT)

5.1 For the assessment of the present claim 44 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to method of doing business.

6. Further comments

6.1 Dependent claim 4 relates to the use of "embryonic stem cells or multipotent adult progenitor cells", therefore encompassing the use of cells originating from human embryos. By consequence the scope of claims 1-54 encompasses the use of human embryos for industrial and/or commercial purposes.

Applicant's attention is drawn to the fact that, upon entry into the regional phase, patentability of claims relating to human embryos may underlie restrictions based on moral grounds. The EPO, for example, does not recognize as patentable the subject-matter of claims to the cloning of human beings, the modification of the germ line identity of human beings and the use of human embryos for industrial or commercial purposes (Article 53(a) and Rule 23d EPC).

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June 15, 2005
[1059198]
PS/SG

File No.:	*Our Reference:*	*Owner/Applicant:*
PCT/EP2004/007529	AX02A03/P-WO	Axiogenesis AG

International Application "Secreted Proteins As Markers For Cell Differentiation" / Axiogenesis AG et al.

This is in response to the Written Opinion drawn up in accordance with Rule 43*bis*.1 PCT issued with the international search report on March 16, 2005, and to be considered to be a Written Opinion of the International Preliminary Examining Authority ("IPEA").

Herewith, a demand for international preliminary examination is made according to the enclosed form PCT/IPEA/401. The prescribed fees in the amount of € 1659.00 are to be debited from our deposit account No. 2800 0980; see also the enclosed Annex to form PCT/IPEA/401.

Furthermore, we enclose herewith an amended set of claims 1 to 52, in triplicate, which should form the basis for the international preliminary examination.

In the following, we would like to comment on the observations raised in the Written Opinion. In doing so, we will refer to the enclosed new set of claims.

1. Amendments to the claims

1.1 Amended claim 1 corresponds to original claim 1 with the amendment that in section (b) the feature "quantifying the differentiated cells by" has been incorporated in order to more clearly indicate the gist of the present invention. The amendment is supported by the description of the application as filed, for example at page 13, lines 3 to 9 and by Example 3.

1.2 Claims 2 to 12 correspond to original claims 2 to 12.

1.3 Amended claim 13 corresponds to original claim 13, wherein the same amendment has been effected as for claim 1; see section 1.1, supra.

1.4 Amended claim 14 corresponds to original claim 14 with the amendment that the claimed cell now mandatory comprises the reporter gene construct of claim 13.

1.5 Claims 15 to 19 correspond to original claims 15 to 19.

1.6 Amended claim 20 corresponds to original claim 20 except for an editorial amendment for the sake of clarity, i.e. the term "reporter gene" has been replaced by the term "reporter gene construct" because of its antecedent basis in claim 13.

1.7 Claim 21 corresponds to original claim 21.

1.8. Amended claim 22 corresponds to original claim 22 but has been redrafted as a true use claim.

1.9 Claims 23 to 41 correspond to original claims 23 to 41.

1.10 Amended claim 42 corresponds to original claim 42 with the amendment that the term "for example" has been deleted and appropriate editorial amendments have been carried out for the sake of clarity.

1.11 Amended claim 43 corresponds to original claim 43 with the amendment that the term "business" has been deleted in line 1 of the claim for the sake of clarity.

1.12 Claims 44 to 48 correspond to original claims 44 to 48.

1.13 Amended claim 49 is based on the subject matter of original claims 49 and 52.

1.14 Amended claim 51 corresponds to original claim 53.

1.15 Amended claim 52 corresponds to original claim 54 with the amendment that the use of the subject promoter region has been further defined in accordance with the methods of the present invention.

It is respectfully submitted that the effected amendments do not introduce new matter but merely have been effected in order to more clearly characterize the claimed subject matter or to correct obvious errors.

2. Clarity (Article 6 PCT)

2.1 Claims 13 to 21, 42, 45 and 46

In section 4.1 of item VIII of the Written Opinion it is observed that the subject matter of claims 13 to 21, 42, 45 and 46 is defined by reference to the method claims instead by features of the products themselves and therefore, according to the Authority, could possibly leave the reader in doubt as to the real scope of the claims.

It is respectfully submitted that this observation is unjustified.

Because of their reference to the preceding claims, the product claims impart the technical features as defined in the claims they are dependent on. For example, claim 13 relates to a reporter gene construct comprising a recombinant nucleic acid molecule as defined in any one of claims 1 to 12, i.e. comprising a reporter gene encoding a product that is secreted upon cell differentiation as recited in claim 1, and which is further defined in dependent claims 2 to 12.

Therefore, the person skilled in the art will immediately and unambiguously recognize without any doubt the properties of the claimed products and thus the subject matter for which protection is sought in claim 13.

The same line of arguments mutatis mutandis applies to claims 14 to 21, 42, 45 and 46.

As a side remark it is noted that the applicant of course could also have drafted the embodiments encompassed by the subject matter of claims 13 to 21, 42, 45 and 46 as a separate set of independent and dependent claims. However, this measure would have resulted in an excessive number of more than 100 claims. Instead, the applicant has

aimed at keeping the claims clear and concise, which should be acknowledged by the Authority.

For the above reasons, it is submitted that the contested claims meet the requirements of Article 6 PCT.

2.2 Number of claims

In section 4.2 of item VIII of the Written Opinion the Authority objects to the number of independent claims.

However, as will also be evident from the explanations given below, the present invention is based on the surprising finding that secreted reporter molecules have unexpected advantages over the use of state of the art reporter proteins in stem cell technology. Hence, the present invention provides a novel genetic marker system which is particularly suited for monitoring differentiation and developmental processes of living cells in both a qualitative and quantitative manner, and therefore can be used for various applications of stem cell technology, for example toxicity tests, drug screening and of course basic developmental research. Thus, due to the generality of the present invention, there are several kinds of aspects the applicant must be allowed to claim. Furthermore, in view of the manifold techniques in molecular and cell biology various embodiments can be performed by the person skilled in the art in order to put the invention into practice. Accordingly, applicant must also be allowed to claim these various embodiments.

In summary, applicant holds the view that the number of claims simply reflects the nature and field of the invention, and is commensurate with the contribution to the art by making the teachings of the present invention available to the public.

2.3 Claims 37 to 39

In section 4.3 of item VIII of the Written Opinion, claims 37 to 39 are objected for alleged lack of clarity.

However, it is respectfully submitted that this objection is unfounded.

The wording of claims 37 to 39 is just a concise characterization of the claimed method recited in the preamble of these claims since their actual process steps are virtually identical to those of the methods of any one of claims 23 to 36 except for the more specific purpose the method is used for, i.e. not for generally obtaining and/or profiling a molulator of cell differentiation but more specifically for obtaining and

manufacturing a drug (claim 37), an agent which supports wound healing and/or healing of damaged tissue (claim 38) and for determining toxicity (claim 39).

In view of this explanation, it should be acknowledged that claims 37 to 39 clearly define their subject matter in accordance with Article 6 PCT.

2.4 Claim 42

In section 4.4 of item VIII of the Written Opinion, original claim 42 had been objected because of the expression "for example" in the claim.

This objection does no longer apply to amended claim 42; see section 1.10, supra.

2.5 Claim 43

In section 4.5 of item VIII of the Written Opinion, original claim 43 was objected since according to the Authority it does not relate to a method of conducting business as recited in the preamble of the claim.

Also this objection does no longer apply in view of the amendment to claim 43; see section 1.11, supra.

3. Novelty (Article 33(2) PCT)

3.1 Claims 1, 2, 5 to 7, 10, 13, 14, 16, 17, 20, 32 to 36 and 49

In section 2.1.1 of item V of the Written Opinion it is argued that claims 1, 2, 5 to 7, 10, 13, 14, 16, 17, 20, 32 to 36 and 49 are allegedly not novel over US patent application US2003/008836 A1 (D1), since according to the Authority this document teaches that the expression of the target gene α-gal which is secreted in the culture medium can be used in order to monitor differentiation of myoblasts into mature myotubes. In this context, reference is made to paragraphs [0053] to [0057] of D1.

However, it is respectfully submitted that the assessment of the teaching of D1 in relation to the claimed invention is not correct.

First of all, as already stated in the abstract of D1, this document relates to methods of medical treatment and vaccination rather than to methods of monitoring cell differentiation as claimed for example in claim 1 of the present application. Accordingly, the transgene α-gal in D1 is used as a therapeutic protein and not as a diagnostic means; see D1 for example at paragraphs [0023] and [0062].

Second, the observations in D1 on the effects of muscle-specific regulatory elements, i.e. the statement in paragraph [0053] that "[g]ene expression can be measured at any time point during differentiation process" for the comparison of the activity of regulatory elements before and after the activation of muscle-specific genes must not be confused with the method of the present invention for determining the status of a differentiating cell, which is based on the quantification of differentiated cells during or after the differentiation process by determining the amount or activity of a secreted reporter gene product.

Furthermore, from the description in D1, for example at paragraph [0053] it is clear that the experiments described in sections [0052] to [0056] are aiming at investigating the effect of the muscle specific regulatory elements with the view of their usefulness for the production of α-gal from muscle in vivo; see D1 for example at paragraph [0062] to [0066].

In contrast, the method of the present invention is for the monitoring of cell differentiation, i.e. determining the status of the cell.

Third, even if the human α-gal protein as used in D1 is considered as a reporter gene product it does not meet the functional requirements recited in claim 1 of the present application. This is because once secreted α-gal does not remain in the culture medium, but is taken up by cells such as fibroblasts; see D1 e.g. at paragraph [0057] and [0058]. However, the claimed method of the present invention requires that the reporter gene product is truly secreted into the cell culture. Otherwise it would not be possible to quantify the differentiated cells, since the amount or activity of the reporter molecule in the cell culture medium would not correspond to the actual number of differentiated cells and/or the status of differentiation; see also the application as filed at page 20, lines 24 to 34.

In summary, D1 neither relates to a method of monitoring cell differentiation as claimed in claim 1 of the present application nor does it provide the necessary means and teaching for doing so.

For this reason, claim 1 is novel over D1.

The same mutatis mutandis applies to claims 2, 5 to 7, 10, 13, 14, 16, 17, 20, 32 to 36 and 49 which are directly or indirectly dependent on claim 1. With respect to claim 13 it should be noted that this claim has been further characterized by the feature of being useful for quantifying differentiated cells in accordance with amended claim 1; see also

section 1.3, supra. Due to dependency and the amendment to claim 14, see section 1.4, supra, this feature is also imparted by claim 14 and the claims dependent on claims 13 and 14.

In summary, the claimed invention is novel over D1.

3.2 Claims 49 to 51 and 54

In section 2.1.2 of item V of the Written Opinion, the present Authority argues that the subject matter of claims 49 to 51 and 54 might be anticipated by documents Müller et al., FASEB J. 14 (2000), 2540-2548 (D2); Wobus et al., Circulation 92 (1995), I-114; Wobus et al., J. Mol. Cell. Cardiol. 29 (1997), 1525-1539 (D4) and Klug et al., J. Clin. Invest. 98 (1996), 216-224 (D5).

However, this observation does not apply to amended claims 49 to 52.

In particular, the present Authority already acknowledged that the vector of original claim 52 and 53 is novel. Since the additional feature in original claim 52 has been incorporated into claim 49; see section 1.13, supra, amended claim 49 as well as its dependent claims 50 to 52 are novel for the same reasons as original claims 52 and 53.

3.3 Claim 22

In view of the amendments to claim 22; see section 1.8, supra, the observations raised in section 2.1.3 of item V of the Written Opinion do no longer apply.

4. Inventive step (Article 33(3) PCT)

In section 3 of item V of the Written Opinion the claims are objected for alleged lack of inventive step.

However, it is submitted that this objection is largely based on the incorrect assessment of document D1 in relation to the claims contested in section 2 of item V of the Written Opinion as has been explained before.

4.1 The technical problem underlying the present invention

As stated in the present application, the problem underlying the present invention is to provide a reliable and highly sensitive ES cell based assay for drug and toxicity screening.

This problem has been solved by the novel and inventive concept of the present

invention to monitor stem cell derived differentiating cells by employing a reporter gene construct, wherein the reporter molecule is expressed and secreted upon cell differentiation. The examples of the present application demonstrate that the problem has been solved in accordance with the claimed invention. In particular, Example 3 demonstrates that the reporter system for the first time provided by the present invention is capable of monitoring the concentration–dependent effect of retinoic acid on differentiating stem cells. More specifically, as described in the present application at page 45, last paragraph, it could be shown that the reporter activity is proportional to the amount of differentiated cells consistent with a corresponding experiment described in Seiler et al., ALTEX 19 (2002), Supplement 1, wherein however the amount of cardiomyocytes was determined by antibody staining of α-actinine and sacromeric myosin heavy chain and subsequent flow cytometry, which of course is quite laborious.

Accordingly, the method of the present invention is indeed suitable for qualifying and quantifying differentiating cells.

4.2 The closest prior art

The closest prior art may be seen in D4, which according to this Authority discloses a method for monitoring cell differentiation.

As correctly assessed by the Authority, D4 describes cardiomyocyte differentiation of embryonic stem cells containing the lacZ gene under the control of the MLC-2v promoter region. As shown in figure 1 and described in the discussion section of D4 the β-galactosidase reporter gene was used as a molecular marker for the process of ventricular specification during in vitro cardiogenesis.

In view of D4, the problem to be solved by the method of the present invention can be considered as the provision of an improved method for the detection and quantification of cells undergoing differentiation.

The solution to said problem as characterized in claim 1 may be framed in accordance with the statement of the present Authority, i.e. to use a different reporter gene which product is released in the culture medium as has been exemplified with secreted alkaline phosphatase (SEAP).

In the last three paragraphs of section 3.1.2 of item V of the Written Opinion, the Authority alleges that the person skilled in the art when confronted with the above stated problem would turn to document Bronstein et al., Biotechniques 17 (1994), 174-177, since this document discloses the advantages of using SEAP as a reporter

gene. Furthermore, according to the Authority US application US2002/042096 A1 (D7) would further support the use of the SEAP reporter system.

Therefore, according to the Authority the person skilled in the art having knowledge of D4 and D6 would have solved said technical problem without exercise of an inventive skill.

It is respectfully submitted that the above observations are unjustified.

4.3 The claimed invention is not obvious

First of all, in order to render a claimed invention obvious the question has to be answered whether a person skilled in the art in view of the prior art such as D4 would have had an incentive to deviate from the teaching of the prior art and to try and use reporter gene products in ES cell technology other than hitherto disclosed for this purpose.

As will be discussed below, the person skilled in the art did and would have not.

In view of D4 the mindset of the person skilled in the art at the relevant time was that the β-galactosidase reporter gene was so to say the gold standard for molecular markers for the process of differentiation; see for example D4 in the discussion section at page 1536, left column, lines 31 to 43. There is not even the slightest hint in D4 to consider any other reporter gene at all.

This is also consistent with the pertinent publications at the relevant time; see also the documents cited in the international search report defining the general state of the art. For example, Metzger et al., Circulation Research 78 (1996), 547-552, also used the lacZ reporter gene for monitoring ES cell differentiation; see e.g. the abstract of Metzger et al.

Likewise, Rohwedel et al., Toxicology In Vitro 15 (2001), 741-753, when discussing future prospects of ES cell technology in cytotoxicity and embryotoxicity studies emphasize the use of the lacZ system for reporter gene expression in genetically engineered ES cells; see Rohwedel et al. at page 749, left column, second full paragraph. The only alternative considered is the use of the green fluorescent protein (GFP). However, secreted reporter gene products such as SEAP are not considered.

In this context, it is worthwhile to note that document D6 describing reporter gene assays for the detection of the GUS and SEAP gene products was published already in

1994, i.e. well before the prior art relating to ES cell differentiation and uses thereof such as D4. Thus, the authors of D4 as well as Metzger et al. and Rohwedel et al. could have already considered the use of a secreted reporter gene product such as SEAP. However, as is evident from the prior art they did not. In contrast, if considering reporter gene products other than β-galactosidase, it was rather tried to establish novel systems such as the use of GFP; see Rohwedel et al., supra, and international application WO02/051987 also cited in the international search report.

With respect to document D7 it is to be noted that this US application does not relate to ES cell technology and mentions the use of the SEAP reporter gene construct in context with already differentiated and established cell lines such as Jurkat-T cells; see, e.g., example 32 referred to by the Authority. D7 neither teaches nor suggests the use of the SEAP reporter in ES cell technology. To the contrary, as can be inferred from paragraph [1153] the SEAP reporter has merely been used out of idle curiosity and it is the stated that "Clearly, however, any reporter molecule can be used instead of SEAP".

Accordingly, even from D7 the person skilled in the art could not have gathered that SEAP is a preferred reporter molecule to be used, let alone in stem cell technology.

This makes already clear that in view of the prior art the person skilled in the art had no incentive to deviate from the teaching of for example D4 and to consider secreted reporter molecules for methods of monitoring ES cell differentiation.

In further support of our arguments, we herewith submit a copy of international application WO97/01644 which may be considered as an expert opinion, in particular in view of the fact that the inventors, i.e. Anna Wobus and Wolfgang-Michael Franz are leading experts in the field of ES cell technology and in vitro tests using genetically engineered ES cells; see also D2, D3, D4 and Rohwedel et al. which are all authored or co-authored by at least one of the inventors of WO97/01644. Also in this document, the reporter gene lacZ is described as "the" reporter gene to be used for screening methods which involve in vitro differentiated ES cells; see WO97/01644 for example at page 4, lines 11 to 17.

Thus, if even the leading experts in the art did not consider a secreted reporter gene product system, how could the person of average skill.

4.4 <u>The method of the present invention provides advantages over the method of D4</u>

As has already been discussed in sections 4.2 and 4.3, supra, D4 teaches to use the lacZ reporter gene system in order to monitor cell differentiation and perform toxicity

screening. The necessary preparation of cell lysates in such conventional assay systems results in a high intra- as well as inter-assay variability.

With the use of a secreted reporter molecule in accordance with the method of the present invention, these drawbacks have been overcome.

Furthermore, it could surprisingly be shown that the amount of determined secreted reporter activity is direct proportional to the amount of the target differentiated cells. Such a clear cut correlation between an assay molecular marker and the status or number of differentiated cells could hitherto only be established with the use of native marker genes; see Seiler et al., (2002); supra.

Hence, the person skilled in the art, even if the use of secreted reporter molecules such as SEAP would have been considered, could not reasonably expect the advantageous reliability and power of such reporter gene constructs in ES cell derived assay systems.

For the above reasons, inventive step for the method of claim 1 should be acknowledged. The same mutatis mutandis applies to claims 2 to 52 which directly or indirectly impart the novel and inventive ES cell reporter gene system of the present invention.

5. Requests

With the above explanations and the amendments to the claims, it is submitted that the applicant has met the requirements of the PCT. It is therefore requested that the Authority's objections be withdrawn and that a favourable IPER be issued.

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Enc.
New set of claims 1 to 52 (in triplicate)
Form PCT/IPEA 401 including annex
c/WO97/01644

Claims

1. A method of monitoring cell differentiation comprising:
   (a) culturing cells capable of differentiating into at least one particular cell type containing at least one recombinant nucleic acid molecule comprising a reporter gene encoding a product that is secreted upon cell differentiation, or maintaining a non-human animal comprising such cells, under conditions allowing differentiation of the cells; and
   (b) quantifying the differentiated cells by determining the amount or activity of the reporter gene product either within a body fluid of said transgenic non-human animal or the cell culture medium.

2. The method of claim 1, wherein said recombinant nucleic acid molecule comprises at least one cell type-specific regulatory sequence operably linked to said reporter gene.

3. The method of claim 1 or 2, wherein said cells are or are derived from stem cells.

4. The method of claim 3, wherein said stem cells are embryonic stem cells or multipotent adult progenitor cells (MAPCs).

5. The method of any one of claims 1 to 4, wherein said reporter gene product comprises a secretory leader sequence.

6. The method of any one of claims 2 to 5, wherein said regulatory sequence comprises a promoter and/or enhancer element.

7. The method of any one of claims 1 to 6, wherein said cell type is selected from the group consisting of connecting fibroblasts, stromal cells, endothelial cells, glial cells, neural cells, neuronal cells, hematopoietic cells, smooth muscle cells, skeletal muscle cells, epithelial cells, and cardiac cells.

8. The method of claim 6 or 7, wherein said promoter or enhancer is selected from the group consisting of aMHC, MLC2V, VE-cadherin, Tie-2, Flk-1, Flt-1, GFAP, alpha-

1-tubulin and collagen 2 promoter or enhancer.

9. The method of any of claims 1 to 8, wherein said reporter gene product is secreted alkaline phosphatase (SEAP) or alpha-amylase.

10. The method of any one of claims 1 to 9, wherein said recombinant nucleic acid molecule further comprises a selectable marker expressed by multi- or pluripotent cells.

11. The method of any one of claims 1 to 10, wherein said cells form cell aggregates or tissue-like aggregates derived from different cell types.

12. The method of any one of claims 1 to 11, wherein said cells form embryoid bodies (EBs).

13. A reporter gene construct for monitoring cell differentiation by quantifying the differentiated cells comprising a recombinant nucleic acid molecule as defined in any one of claims 1 to 12.

14. A cell as defined in any one of claims 1 to 12 comprising a reporter gene construct of claim 13, wherein said cell is capable of differentiating into at least one particular cell type.

15. A cell aggregate of at least one cell type obtainable by the method of any one of claims 1 to 12.

16. A tissue obtainable by the method of any one of claims 1 to 12 or comprising cells of claim 14 or a cell aggregate of claim 15.

17. An organ comprising a tissue of claim 16, a cell of claim 14 or a cell aggregate of claim 15.

18. An implant or transplant comprising an organ of claim 17, a tissue of claim 16, a cell of claim 14 or a cell aggregrate of claim 15.

19. A non-human animal comprising a reporter gene construct of claim 13, a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16 or an organ of claim 17.

20. A composition of matter comprising a reporter gene construct of claim 13, a tissue of claim 16, cells of claim 14 or a cell aggregate of claim 15.

21. An array comprising a solid support and attached thereto or suspended thereon cells of claim 14, a cell aggregate of claim 15 or a tissue of claim 16.

22. Use of an apparatus for analyzing the array of claim 21.

23. A method of obtaining and/or profiling a modulator of cell differentiation comprising:

(a) contacting a test sample comprising a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16 or an organ of claim 17 or a non-human animal of claim 19 with a test substance; and

(b) determining the effect of the test substance on the amount of the reporter gene product or activity compared to a control sample or animal.

24. The method of claim 23, wherein said contacting step further includes contacting said test sample or animal with at least one second test substance in the presence of said first test substance.

25. The method of any one of claims 1 to 12 or 23 to 24, wherein a compound known to activate or inhibit the differentiation process is added to the culture medium or animal.

26. The method of any one of claims 23 to 25, wherein the test substance is a therapeutic agent.

27. The method of any one of claims 23 to 26, wherein the test substance is a mixture of therapeutic agents.

28. The method of any one of claims 23 to 27, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

29. The method of claim 28, wherein said collection of test substances has a diversity of about 103 to about 105.

30. The method of claim 29, wherein the diversity of said collection of test substances is successively reduced.

31. The method of any one of claims 23 to 30, which is performed on an array.

32. The method of any one of claims 1 to 12 or 23 to 31, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

33. The method of any one of claims 1 to 12 or 23 to 32, wherein said one or more cells or tissue are contained in a container.

34. The method of any one of claims 1 to 12 or 23 to 33, comprising taking 3 or more measurements, optionally at different positions within the container.

35. The method of claim 33 or 34, wherein said container is a well in a microtiter plate.

36. The method of claim 35, wherein said microtiter plate is a 24 , 96 , 384 or 1586 well plate.

37. A method of obtaining and manufacturing a drug which promotes or inhibits formation of specific cell types comprising the steps of any one of claims 23 to 36, wherein an enhanced or reduced level or activity of the reporter gene product is indicative for the drug.

38. A method of manufacturing an agent which supports wound healing and/or healing of damaged tissue comprising the steps of the method of any one of claims 23 to 37, wherein an enhanced level or activity of the reporter gene product is indicative for said agent.

39. A method of determining toxicity, preferably teratogenicity, embryotoxicity, chronic or acute toxicity of a compound comprising the steps of the method of any one of claims 23 to 37, wherein a reduced or enhanced level or activity of said reporter gene product is indicative for the toxicity of the compound.

40. The method of any one of claims 23 to 39, further comprising modifying said substance to alter, eliminate and/or derivatize a portion thereof suspected causing toxicity, increasing bioavailability, solubility and/or half-life.

41. The method of any one of claims 23 to 40, further comprising mixing the substance isolated or modified with a pharmaceutically acceptable carrier.

42. A kit useful for conducting a method of any one of claims 1 to 12 or 23 to 41, containing a reporter gene construct of claim 13 or a cell of claim 14, and optionally standard compounds, like cell culture media, selection agents, detection agents for the reporter molecule and control samples.

43. A method of conducting a drug discovery comprising:

(a) providing one or more assay systems of any one of claims 1 to 12 or 23 to 41 for identifying a modulator of cell differentiation; and/or

(b) conducting therapeutic profiling of modulators identified in step (a), or further analogs thereof, for efficacy and toxicity in animals of claim 19; and

(c) formulating a pharmaceutical preparation including one or more modulators identified in step (b) as having an acceptable therapeutic profile.

44. A method of conducting a target discovery business comprising:

(a) providing one or more assay systems of any one of claims 1 to 12 or 23 to 41 for identifying modulators of cell differentiation;

(b) (optionally) conducting therapeutic profiling of modulators identified in step (a) for efficacy and toxicity in animals of claim 19; and

(c) licensing, to a third party, the rights for further drug development and/or sales for modulators identified in step (a), or analogs thereof.

45. A modulator of cell differentiation such as growth and tissue formation promoting identified according to the method of any one of claims 23 to 41.

46. A pharmaceutical composition for use in the modulation of cell differentiation comprising a modulator identified according to the method of any one of claims 23 to 41.

47. A method of making a pharmaceutical composition for use in modulating cell differentiation comprising mixing a modulator of cell differentiation identified according to a method of any one of claims 23 to 41 with a suitable diluent or carrier.

48. Use of a reporter gene construct of claim 13, a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16, an organ of claim 17, the implant or transplant of claim 18, a non-human animal of claim 19, the composition of claim 20, an array of claim 21 or the apparatus of claim 22 in drug discovery or pharmacokinetic or pharmacological profiling.

49. A vector comprising the promoter region of the mouse alpha myosin heavy chain gene or of the ventricular myosin regulatory light chain gene, and operably linked thereto a heterologous DNA sequence, which encodes secreted alkaline phosphatase protein (SEAP).

50. The vector of claim 49, wherein said promoter comprises the nucleotide sequence of SEQ ID NO: 1 or 2, or a fragment thereof.

51. The vector of claim 49 or 50 comprising the nucleotide sequence of SEQ ID NO: 3.

52. Use of a promoter region of the mouse alpha myosin heavy chain gene or of the ventricular myosin regulatory light chain gene for the specific expression of heterologous DNA sequences during embryogenesis or cell development in a method of any one of claims 1 to 12 or 23 to 41.